An Outbreak of *Acinetobacter baumannii* Septicemia in a Neonatal Intensive Care Unit of a University Hospital in Brazil

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We studied an outbreak of two multi-drug resistant clones of *Acinetobacter baumannii* in the Neonatal Intensive Care Unit of the Uberlândia Federal University Hospital in Minas Gerais state, Brazil, and we analyzed the contribution of cross-transmission in the rise in infection rates. Eleven neonates who developed multi-drug resistant *A. baumannii* nosocomial infection were matched to 22 neonates who were admitted to the same unit and did not develop an infection during the outbreak period, in order to identify risk factors for infection. Three out of the 11 neonates died. Epidemiological investigation included molecular typing, using pulsed field gel electrophoresis. Prior to the outbreak, from December 2001 to March 2002, no case of infection by this microorganism was diagnosed. Environmental and healthcare worker hand cultures were negative. Nine isolates had similar pulsed field gel electrophoresis patterns and two had another clone. The first clone was brought into the unit by an infected patient who was transferred from another hospital without a history of antibiotic use. The second clone did have its origin clearly defined. Both infected groups led us to conclude that several factors contributed to infection with *A. baumannii*. These factors were: exposure to antibiotics and invasive devices, birth weight ≤ 1500g, age ≤ 7 days and duration of hospitalization ≥ 7 days. Based on logistic regression, infected neonates were more exposed to carbapenem and mechanical ventilation than the control group. Cross transmission between infants contributed to the rise in the rates of multi-drug resistant *A. baumannii* infection.

Key Words: *Acinetobacter baumannii*, molecular epidemiology, outbreak, neonates.

Acinetobacter baumannii is a ubiquitous microorganism that has become an important nosocomial pathogen, particularly in intensive care units (ICUs) [1,2]. Various types of infections are caused by this microorganism, including pneumonias, bacteremias, meningitis and urinary tract infections [3-5]. Although classically described as a nosocomial pathogen in adults, *A. baumannii* is also an important pathogen in neonates hospitalized in ICUs [3]. Most *A. baumannii* outbreaks have been traced to environmental sources, such as patient mattresses, air conditioners and mechanical ventilation equipment [6]. The risk factors associated with nosocomial infections due to this microorganism include mechanical ventilation, surgery, and trauma [7]. Increasing rates of *A. baumannii* infections may be due to lapses in infection-control practices. In these situations, "colonization pressure", which is a function of the proportion of patients already colonized or infected with *A. baumannii*, can affect the likelihood of cross-transmission between patients [8].

Received on 18 April 2005; revised 27 July 2005.

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The Brazilian Journal of Infectious Diseases 2005;9(3):301-309

We describe an outbreak of nosocomial infection caused by two multidrug-resistant (MDR) *A. baumannii* clones in a Neonatal Intensive Care Unit (NICU), involving 11 cases. In addition, to analyzing the contribution of cross-transmission, the mean daily point prevalence of patients with MDR *A. baumannii* infection was quantified, and a case-control study was performed.

Materials and Methods

Clinical setting

The NICU of Uberlândia Federal University Hospital has nine beds, reserved for patients requiring intensive care, such as mechanical ventilation and cardiac support.

Design of the study

We made a case-control study from October 2001 to March 2002; a case was defined as any infant hospitalized in the NICU who developed *A. baumannii* infection (n=11) confirmed by laboratory exam, and other neonates hospitalized during the same period, with no diagnosis of infectious syndrome (n=22) were the controls. The localization of the cases in the incubators was recorded (Figure 1). An individual record was filled out with the patient's demographic data, along with possible predisposing factors, including gender, birth weight, age, duration of hospitalization, gestational age, apgar score at five minutes, incubator care, use of antibiotics, mechanical ventilation and use of a central or peripheral venous catheter.

Microbiological investigation

All the *A. baumannii* samples were inoculated in sheep blood agar and incubated at 35ºC. Typical colonies were examined. The genus was identified by Gram staining, cell and colony morphology, oxidation/fermentation test activity, absence of motility and negative oxidase and positive catalase reactions. The species identification of *A. baumannii* was determined by lactose and glucose fermentation, urease activity and hemolysis of sheep blood [9]. Clinical oropharynx and rectal materials were obtained with swabs of all hospitalized neonates on two occasions (December 2001 and January 2002), during the outbreak. Environmental cultures were taken at these times, including samples from incubators, tap water, sink areas, mechanical ventilator, liquid soap, and air; this last through the exposition of petri plates with blood agar and MacConkey agar. Cultures from the hands of most staff working in the NICU were obtained during the same periods.

The antibiotic susceptibilities of the 11 *A. baumannii* isolates were determined by the disk diffusion method on Mueller-Hinton agar plates, with the following antibiotics: gentamicin, ciprofloxacin, ceftazidime, cefpime, cefpirome, aztreonam, imipenem and ampicillin/sulbactam [10], and the minimum inhibitory concentrations (MICs) of ceftazidime and cefpime were determined by agar dilution technique on Mueller-Hinton agar plates, with the following ceftazidime and cefepime concentrations: 0.5 to 256μg/mL [11]. The genotypic analysis of isolated *A. baumannii* was performed by pulsed-field gel electrophoresis (PFGE). The DNA fragments generated by digestion with the restriction enzyme SmaI were analyzed using the Gene Navigator apparatus (Pharmacia Biotech). The criterium for interpreting PFGE patterns was the number of fragment differences compared to the outbreak pattern, as follows: 0 differences was interpreted as part of the outbreak; 2 to 3 differences was probably part of the outbreak; 4 to 6 differences was possibly part of the outbreak; and ≥ 7 differences was not part of the outbreak [12].

Case-control study

For each patient, the mean daily point-in-time prevalence of neonates with MDR *A. baumannii* infection in the unit was calculated. The final point was the date of the discharge from the unit for cases and controls.

Statistical analysis

Categorical variables were compared by using the likelihood ratio test or, when appropriate, Fisher's exact test. Continuous variables were analyzed by the student's t-test, or the Wilcoxon Rank-Sum Test for non-parametric distributions [13]. To assess the independent effects of significant variables in the univariate analysis, we performed multivariate analysis, using stepwise logistic regression [14].

Results

Description of the outbreak and case characteristics

The outbreak began in October 2001, and it extended until March 2002 (Figure 2); In the previous year, no case of infection by *A. baumannii* was recorded in the NICU. In September 2002, a neonate was observed to be infected by *A. baumannii*; however, the microorganism did not have the same characteristics as the epidemic strain. The index case occurred in the first week of October. It involved a 2760g neonate, coming from the Maternity Nossa Senhora Aparecida, of Iturama, MG; the baby was transferred at 45 hours after birth, in convulsive crisis, with suspicion of meningitis and without report of antibiotic use. During the outbreak, the epidemic strain of *A. baumannii* was isolated from 11 neonates. It was identified from blood culture, and the three neonates that died presented the following characteristics (Table 1): case 3 - 2610g, cardiac patient, maintained in an incubator, use of a central venous catheter, cases 10 and 11, infected with the second clone, premature (1010g), maintained in incubators, breather use and umbilical venous catheter (case 10) and peripheral catheter (case 11). All the infected neonates were treated with imipenem.

During the period of the outbreak, three beds of the NICU had held more than one infected neonate. The unit was not closed to new admissions.

Epidemiology

The risk factors for *A. baumannii* infection are described in the Table 2. On univariate analysis, the following factors were statistically significant: weight £ 1500g, age ≤ 7 days, duration of hospitalization ≥ 7 days, carbapenem use, antibiotic use, use of central venous catheter and mechanical ventilation, when compared to the control group. By conditional logistic regression, the only independent risk factors for developing a nosocomial MDR *A. baumannii* infection were the use of carbapenem (odds ratio, 4.95; 95% confidence interval, 1.51-18.80; p= 0.031) and mechanical ventilation (odds ratio, 3.49; 95% confidence interval, 1.13-15.20; p= 0.047).

Risk factors for *A. baumannii* infection

Cases were exposed to a mean of 19.2 different patients (range, 6-41) in the unit, among whom a mean of 3.1 (range, 0-6) neonates had an MDR *A. baumannii* infection. In contrast, controls were exposed to a mean of 8.8 different patients (range 1-29), of whom an average of 2.9 (range 1-5) neonates had an MDR *A. baumannii* infection. The mean daily point prevalence of neonates with MDR *A. baumannii* infections was significantly greater among cases (28.1 ± 16.9%) than among controls (13.4 ± 7.4%; p = 0.005).

In spite of the outbreak being restricted to six months, there were administrative difficulties in instituting cohort isolation, resulting in the death of three hospitalized neonates, all of them occurring after the detection of the *A. baumannii* epidemic strain in other neonates of the unit.

Although there was surveillance intestinal and oropharynx colonization, and cultures were made from health care workers' hands and several environmental sources at during two periods (December 2001 and January 2002), both during the epidemic (Figure 2), the results were negative.

Microbiological surveillance

All the *A. baumannii* isolates presented the same resistance profile to the antibiotics gentamicin,

Figure 1. Diagram of the neonatal intensive care unit, October 2001, to March 2002.

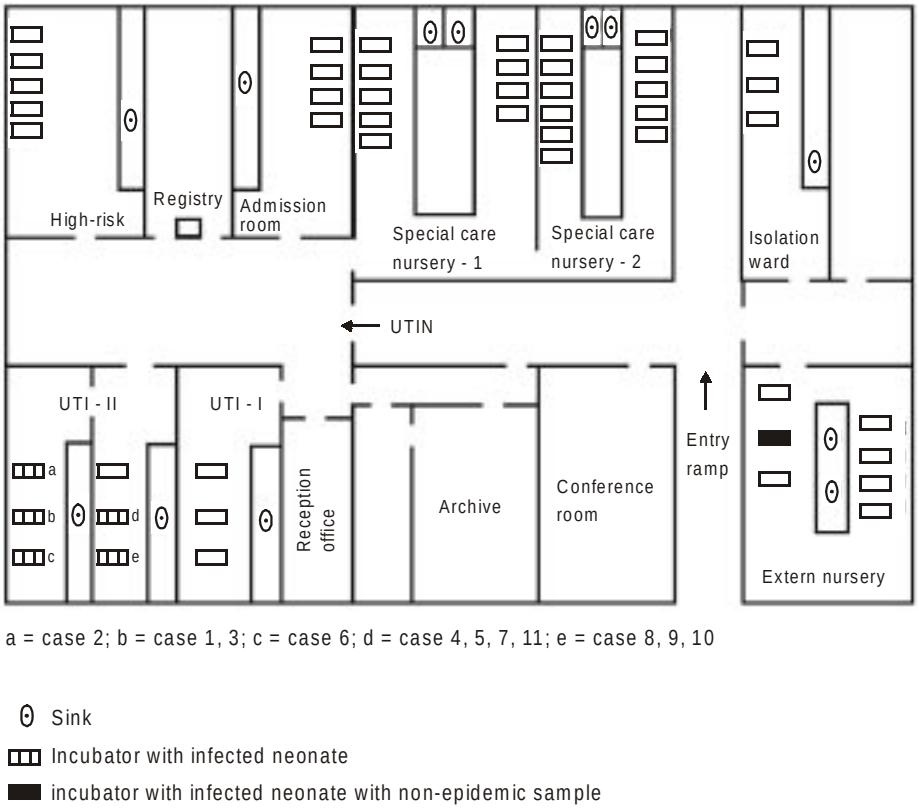


Figure 2. Distribution of neonatal intensive care unit case patients. Uberlândia Federal University, Uberlândia, Brazil, September, 2001 to September, 2002.

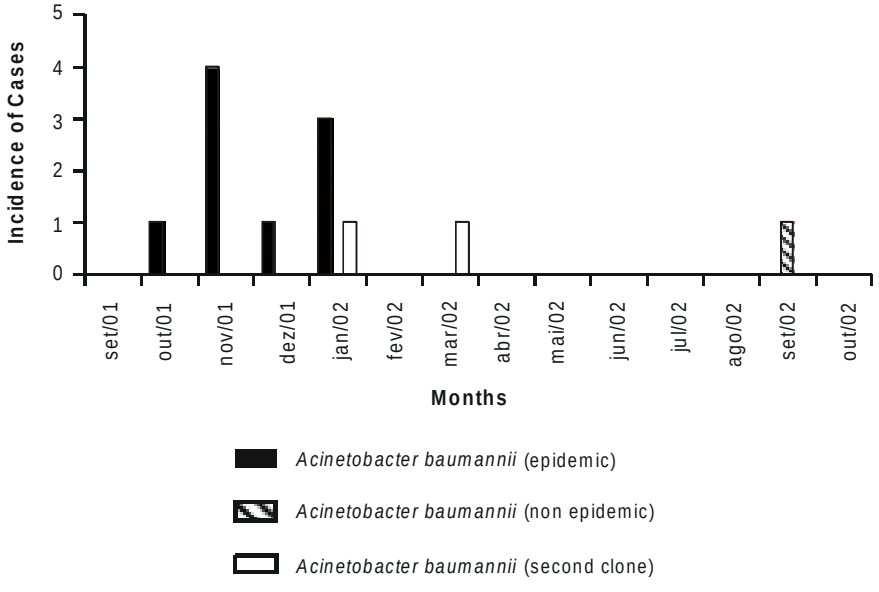
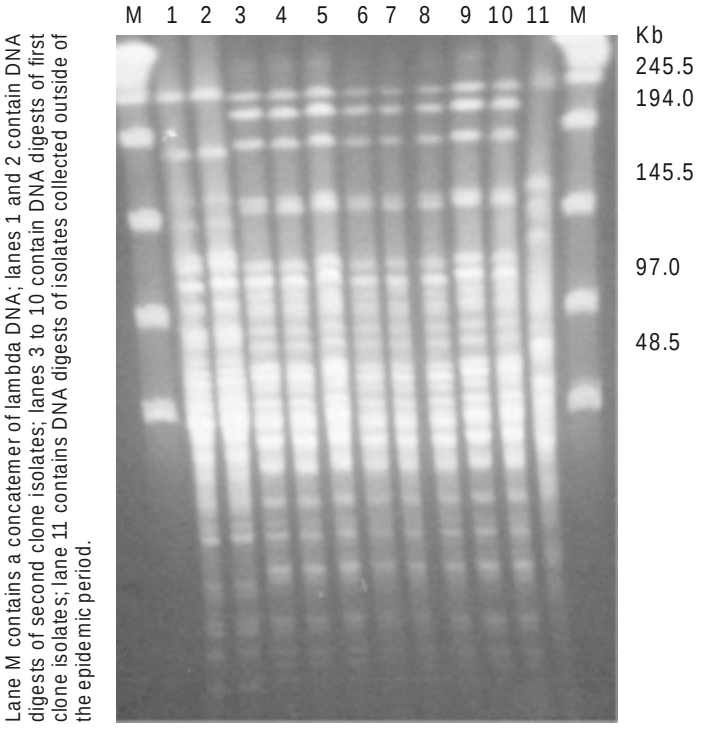


Figure 3. Representative pulsed field gel electrophoresis patterns from 11 strains of the *Acinetobacter baumannii*.



ciprofloxacin, ceftazidime, cefpime and aztreonam, with susceptibility only to ampicillin/sulbactam and imipenem. The epidemic strain was resistant to ceftazidime and cefpime by the agar dilution test. Production of ESBL was detected in six of the 11 samples (data not shown).

Analysis of the *A. baumannii* strains with PFGE showed two clones, both with band profiles different from the strain of this microorganism that was isolated in the unit after the outbreak (September 2002). The PFGE patterns of the 11 strains of the *A baumannii* isolates are shown in Figure 3; lane M contains concatemers of lambda DNA; lanes 1 and 2 contain DNA digests of second clone isolates; lanes 3 to 10 contain DNA digests of first clone isolates; lane 11 contains DNA digests of isolates from outside of the epidemic period.

Discussion

The bacteria *A. baumannii* has emerged as an important nosocomial pathogen, and outbreaks due to multiply-resistant strains have been difficult to control, especially in ICUs [15]. We have documented an outbreak of MDR *A. baumannii* in an NICU during a six-month period, affecting 11 neonates, with three deaths. To define the risk factors for the acquisition of infection, we compared the case-infant group with a randomly-selected group of control infants; the following factors were significant: weight ≤ 1500g, age ≤ 7 days, duration of hospitalization ≥ 7 days, use of imipenem, use of a central venous catheter and mechanical ventilation. These same risk factors were described by McDonald [3] and Melamed [5].

Although the outbreak caused by *A. baumannii* was traced to a common source, in agreement with prior studies, the environmental reservoirs indicated in those studies (respiratory equipment [2], mattresses [3], suction catheters [2], laryngoscopes [2], gloves [6] and air conditioner [3] were not identified. Though the dissemination of this pathogen is facilitated by its prolonged survival on inanimate surfaces, high colonization rates among hospitalized patients, and frequent contamination of healthcare workers' hands

Table 1. Clinical characteristics of 11 neonates with clinical disease associated with *Acinetobacter baumannii*

Case	Isolation date	Infection	Diagnostic	Invasive procedures	Weight (g)	Incubator	Antibiotic	Evolution
1	10/07/01	sepsis	HMD	UVC	2,760	+	+	survived
2	11/07/01	sepsis	Premature, pulmonary hemorrhage	CVC, CVP, intubation	1,180	+	+	survived
3	11/12/01	sepsis	cardiopathy	CVC	2,610	+	+	died
4	11/12/01	sepsis	HMD, premature	CVP	1,090	+	+	survived
5	11/26/01	sepsis	HMD	CVP	1,340	+	+	survived
6	12/09/01	sepsis	HMD, premature intubation	CVC, CVP,	880	+	+	survived
7	01/01/02	sepsis	HMD, premature	CVC, CVP	2,210	+	+	survived
8	01/17/02	sepsis	HMD, premature	CVP, intubation	2,200	+	-	survived
9	01/24/02	sepsis	HMD	CVC, intubation	1,210	+	+	survived
10	01/25/02	sepsis	HMD (anoxia)	UVC, intubation	800	+	+	died
11	03/10/02	sepsis	HMD	CVP, intubation	1,010	+	-	died

HMD = hyaline membrane disease; CVC = central venous catheter; UVC = umbilical venous catheter.

Table 2. Risk factors for *Acinetobacter baumannii* infection in neonates

Risk factor	Cases N=11		Controls N=22		Unadjusted OR	p Value
	N	%	N	%		
Weight at birth						
>1500g	4	3.6	17	77.3	0.17 (0.02-1.03)	0.05*
≤1500g	7	69.6	5	22.7		
Sex						
Female	8	72.3	7	31.8	5.71 (0.93-39.7)	0.06
Male	3	27.3	15	68.2		
Age						
>7 days	7	63.6	21	95.4	0.08 (0.00-1.06)	0.03*
≤7 days	4	36.4	1	4.6		
Weeks of gestation						
26-39	5	45.4	4	18.2	3.75 (0.59-25.79)	0.12
30-33	4	36.4	6	27.3	1.52 (0.25-9.29)	0.69
34-36	2	18.2	12	54.5	0.19 (0.02-1.30)	0.07
Hospitalization duration (≥7 days)	10	90.9	6	27.3	26.67 (2.41-692.79)	<0.001*
Apgar at 5 minutes						
0-4	4	36.4	5	22.7	1.94 (0.31-12.50)	0.43
5-7	1	9.1	7	31.8	0.21 (0.01-2.31)	0.21
8-10	6	54.5	10	45.5	1.44 (0.27-7.87)	0.90
Incubator	11	100.0	20	20.9	indefinite	0.54
Antibiotic use	11	100.0	12	54.5	indefinite	0.01*
Carbapenem use	11	100.0	0	0	indefinite	<0.001*
Utilization of CVC	5	45.4	1	4.5	17.50 (1.42-486.05)	<0.001*
Mechanical ventilation	8	72.7	1	4.5	56.00 (4.07-1781.29)	<0.001*
Daily point prevalence of patients with MDR *A. baumannii* infections (%)	0-20	4	15		4.31 (1.46-13.00)	0.002*
	21-40	5	7			
	41-60	2	0			
	61-80	0	0			
	81-100	0	0			
Exposure to patients with an MDR *A. baumannii* infection during unit admission (number patients)	0	1	0		2.00 (0.26-15.60)	0.43
	1-2	3	11			
	3-4	5	4			
	5-6	2	7			
	7-8	0	0			

* P=0.05 (statistically significant); OR = odds ratio; MDR = multidrug resistant; CVC = central venous catheter.

have been observed. In our study, all environmental and hand cultures were negative for MDR *A. baumannii*. Another unusual aspect was that the index case was a neonate with suspicion of meningitis, transferred from a small nearby city, without indication of the use of any antibiotics, with a blood culture made before instituting antibiotic therapy. All documented infections in this outbreak were septicemias in neonates using central or peripheral venous catheters. The mortality associated with this infectious syndrome in neonates ranges from 13.9 to 83% [2,3], while we found 27% (3 of 11).

The potential for acquisition of MDR *A. baumannii* from another neonate was considered and quantified by calculating the mean daily point prevalence of neonates with MDR *A. baumannii* infections to which each neonate was exposed to. Several studies have found that the prior use of third-generation cephalosporins (especially ceftazidime), fluorquinolones, and carbapenems is associated with the subsequent development of MDR *A. baumannii* [1,8,12,16,17]. During the period of March to September 2001, preceding the outbreak that we monitored, an increase in the consumption of this cephalosporin was observed due to an outbreak of *Pseudomonas aeruginosa* (data not shown).

The *A. baumannii* isolates were resistant to third and fourth generation cephalosporins, and six out 11 were ESBL producers. Other mechanisms of acquiring resistance have been described for *A. baumannii* resistant to cephalosporins and carbapenems, including altered penicillin-binding proteins, the presence of metallo beta lactamases, and the loss of porins [17].

Genotyping by PFGE has been shown to be a powerful tool for a better understanding of the epidemiology of nosocomial infection [1,5]. We found two clones of *A. baumannii* isolated from the blood of neonates hospitalized in our NICU during the study period. Surveillance made during nine-month periods prior and posterior to the outbreak, demonstrated that this microorganism was not present in the unit.

In conclusion, based on a thorough clinical epidemiological investigation, with the use of molecular epidemiology technology, we can conclude that, in many cases, when a contaminated environmental source cannot be identified, the increase in the infection rate due to a particular pathogen may be due to lapses in infection-control measures, resulting in an increase in cross-transmission between patients. Containment of the outbreak was achieved by strict hygienic measures and cohort nursing of the infected infants.

Acknowledgements

We thank Josiane Ferreira for technical assistance during molecular assays.

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